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Alzheimer's disease due to loss of function: A new synthesis of the available data

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Abstract

Alzheimer's Disease (AD) is a highly complex disease involving a broad range of clinical, cellular, and biochemical manifestations that are currently not understood in combination. This has led to many views of AD, e.g. the amyloid, tau, presenilin, oxidative stress, and metal hypotheses. The amyloid hypothesis has dominated the field with its assumption that buildup of pathogenic β-amyloid (Aβ) peptide causes disease. This paradigm has been criticized, yet most data suggest that Aβ plays a key role in the disease. Here, a new loss-of-function hypothesis is synthesized that accounts for the anomalies of the amyloid hypothesis, e.g. the curious pathogenicity of the $A\beta_{42}/A\beta_{40}$ ratio, the loss of Aβ caused by presenilin mutation, the mixed phenotypes of APP mutations, the poor clinical-biochemical correlations for genetic variant carriers, and the failure of Aβ reducing drugs. The amyloid-loss view accounts for recent findings on the structure and chemical features of Aβ variants and their coupling to human patient data. The lost normal function of APP/Aβ is argued to be metal transport across neuronal membranes, a view with no apparent anomalies and substantially more explanatory power than the gain-of-function amyloid hypothesis. In the loss-of-function scenario, the central event of Aβ aggregation is interpreted as a *loss of soluble, functional monomer Aβ* rather than *toxic overload of oligomers*. Accordingly, new research models and treatment strategies should focus on remediation of the functional amyloid *balance*, rather than *strict containment* of Aβ, which, for reasons rationalized in this review, has failed clinically.

***Keywords*: β-amyloid, loss of function, Alzheimer's disease, protein misfolding, metal transport**

Introduction

As this is written, every day brings approximately 4000 new cases of Alzheimer's Disease (AD) to the world: Approximately 28−33 million people world-wide suffer from AD[1][2]; in 2010, 21−25 million AD cases were estimated[3] (60−70% of 35.6 million dementia cases), suggesting an annual growth of ~1.5 million cases.

The disease is terrifying to patients and relatives as the gradual loss of memory and identify leads to a slow but steady degeneration of life quality, and the societal costs of AD constitutes a major challenge to healthcare budgets[4][5]. Current treatments delay disease progression by only some months[4][6], and the recent failures of clinical trials of leading drug candidates have been a major concern[7][8][9][10][11][12][13].

AD first manifests slowly, starting with mild cognitive impairment[14][15][16], then leads to gradual loss of cognitive skills, identity, and activity[17][18]. The loss of neurons takes place primarily in the cerebral cortex and in the hippocampus[19]. Definitive AD diagnosis includes deposits of senile plaques outside the neurons and neurofibrillar tangles inside them as two major hallmarks of this disease[16][18]. The senile plaques consist of oxidized and metal-bound β-amyloid (Aβ) peptides organized as regular β-sheet-structured fibrils[20][21]. The neurofibrillar tangles consist primarily of phosphorylated tau protein[22][23]. AD patients also bear marks of massive molecular oxidative stress[24], impaired glucose utilization[25][26], diabetes-like pathologies[26][27], and imbalances in metal ion levels, such as calcium[28][29][30], iron[31][32], zinc[19][33][34], and copper[35][36].

The failure to efficiently treat AD directly relates to the fact that AD is a complex, multi-factor late-onset neurological disease: Approximately 95% of AD cases occur with no apparent family history as so called "sporadic AD" (SAD); only about 5% of emerging AD cases can be related to a family history, i.e." familial AD" (FAD)[37]. The complexity is further reflected in the very broad clinical spectrum of the disease with multiple cognitive, as well as histopathological manifestations[16], the

highly variable clinical features and survival times[9], and the notable fact that age, not any particular gene or event, is the main risk factor of the disease[38], whereas a large range of minor genetic, life-style, and environmental risk modifiers contribute to overall disease risk[39][40].

This complexity and the many histopathological aspects of the disease have produced a range of hypotheses on the underlying causes of AD (see **Figure 1**): Focus on the biomolecular changes have led to several "post-translational" hypotheses on the causes of AD focusing on these features[19], e.g. the tau[41][42][43][44], metal ion[45][46] and oxidative stress[47][48] hypotheses. These hypotheses supplement the genetics-supported amyloid and presenilin hypotheses to be discussed below and the early cholinergic hypothesis that emphasized the direct impairment of pathways involving acetylcholine-rich neurons[49][50]. As will be discussed in this review, these hypotheses are *not* contradictory but rather convey specific pieces of information that, when put together in context, provides a unique and consistent picture of the true nature of the disease.

In terms of genetic risk, FAD has been related to variations in the genes coding for the amyloid precursor protein (APP)[51] and the presenilin isoforms PSEN1 and PSEN2[52][53][54][55][56]. Thus, APP and PSEN have been the central frameworks of AD research, leading to two "gene-centric" hypotheses of AD etiology: the amyloid hypothesis[20][57][58] and the presenilin hypothesis[59][60]. In addition, the apolipoprotein E ε4 allele (ApoE4) increases risk by several times for heterozygote carriers and by up to 15-fold for homozygotes[61][62][63]. The sorting protein-related receptor (SORL1 or LR11) has been suspected of being involved in AD for more than a decade[64], and was found to be downregulated in AD [65]. It relates to APP and Aβ trafficking, and lipid metabolism (it works as a ApoE receptor), putting it in possible relation to other genetic risk factors[66]. In 2007, its gene SORL1 was identified as a likely risk factor of AD[67][68]. Genome-wide association studies (GWAS) have identified a range of other variations that may increase risk of AD[69][70], e.g. *GAB2*, *GALP*, *TNK1*, *TREM2*, *PICALM*, and *CLU*[54][70][71][72][73].

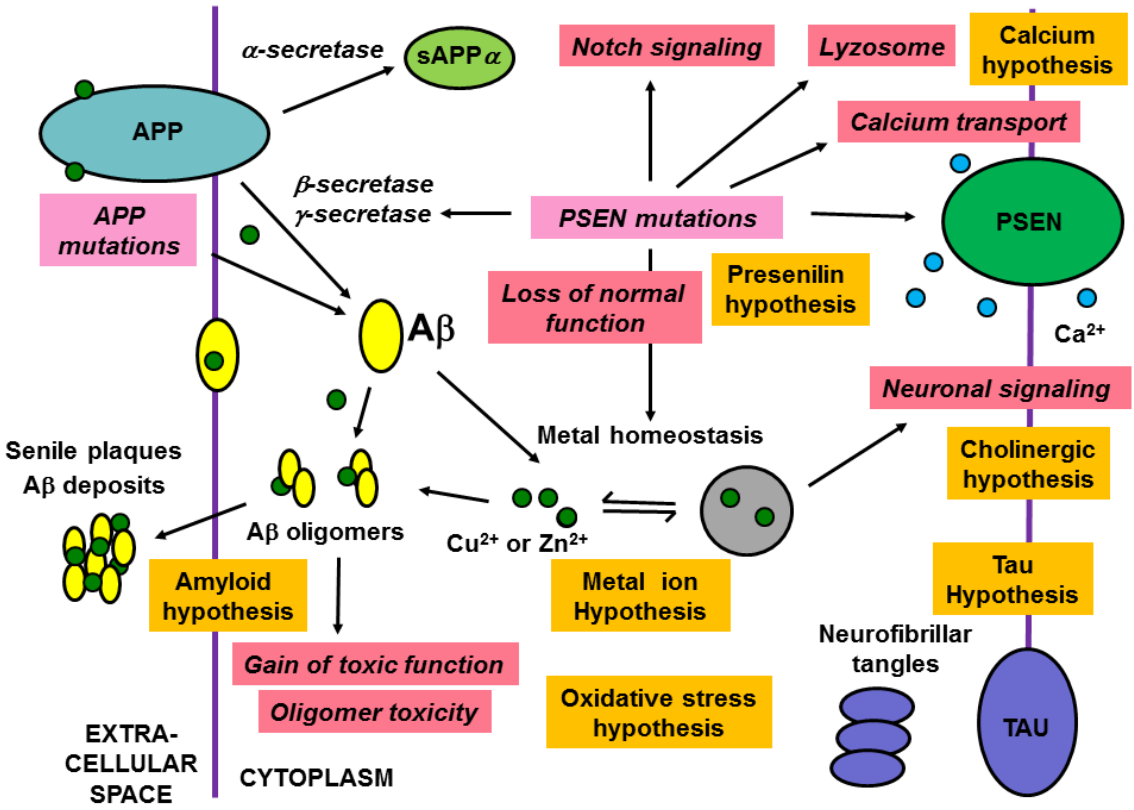

Figure 1. Simplified overview of the biochemistry of Alzheimer's disease. Previously proposed mechanistic hypotheses are shown in orange and key pathologies in red. APP, PSEN, and Aβ are shown as light blue, green, and yellow, respectively, and metal ions are shown as small green or blue circles. The gain-of-function amyloid hypothesis dominates the left of the figure, and alternative hypotheses are located to the right.

Some established life-style-related risk modifiers are body mass index[74][75][76][77], smoking[78] [79], alcohol intake[80][81][82], diabetes[27][83], hypertension[84], depression[85][86], and overall (both mental and physical) inactivity[87][88][89]. Some risk modifiers reduce risk of AD, including mental and physical activity (e.g. education)[39][90][88], anti-oxidant and vitamin rich diets[39][91][92][93][94]. Several of these risk modifiers probably correlate, so that in summary, an active, healthy, and positive life style reduces risk of AD.

The amyloid hypothesis: Gain of toxic Aβ function

The senile plaques characteristic of AD have been a natural starting point for understanding the disease: The parallel of protein deposits to Creutzfeldt-Jakob disease led to the original hypothesis that these plaques in AD might be pathogenic[95]. When these plaques were found to contain large amounts of Aβ peptides, the basis for the amyloid hypothesis was established[96]. Representative structures of the Aβ monomer in water and the β-sheet-structured aggregates characteristic of senile plaques are shown in **Figure 2 A** and **Figure 2B**, respectively. Subsequently, it was found that Aβ is produced from APP by the action of β- and γ-secretases[97][98], and moreover, that PSEN is the catalytic unit of the γ-secretase[99][100][101], which also cleaves other important substrates such as Notch[102][103]. Thus, the two major genetic risks of FAD, PSEN and APP, suggested a role of Aβ and supported the dominating paradigm of AD, the amyloid hypothesis[20][57][104].

The amyloid hypothesis is a "gain-of-function" hypothesis, i.e. it proposes that Aβ attains a toxic function in the brain that leads to disease[11][57]. In its early form, referred to as the "amyloid cascade hypothesis", it was a *quantitative* gain-of-function hypothesis, i.e. it was assumed that the steady state levels of Aβ were toxic in themselves, and that these levels were increased gradually during progression of AD[20][104]. This "overload" etiology would provide an explanation for the vast Aβ deposits in brains that typically represent several years of total Aβ production[105].

However, various observations were not consistent with a cascade of quantitative build-up as the main culprit of AD: First, neuro-degeneration and cognitive decline does not correlate significantly with plaque Aβ load[6,11], implying that the plaque load is not a characteristic feature of disease severity. Neuron loss generally begins in specific parts of the brain (e.g. hippocampus) although Aβ is found across the brain[106][107]. Also, a substantial fraction (perhaps 20−40%) of cognitively normal older people have Aβ plaques[108] that would satisfy commonly applied AD diagnosis

criteria[109][110]. It has been suggested that these deposits in normal people represent pre-clinical disease states[111], but more likely, the plaques are not involved themselves in the pathogenesis, because they are insoluble and outside the cells: Instead, intracellular soluble oligomers of Aβ, not fibrils as found in plaques, are the most toxic forms of the peptide[112][113][114][115][116]. Plaques might then in fact be indicators of a protective pathway whereby Aβ is exported from cells and aggregates into fibrils, to reduce the burden of intracellular oligomers[117][118].

FAD-related mutations in PSEN1 often do not lead to higher, but lower Aβ levels measured in cultured cells[97][119], which is not consistent with quantitative gain-of-function[9]. The Aβ peptides produced by cleavage of APP vary in length, with the two dominating isoforms, $A\beta_{40}$ and $A\beta_{42}$, having 40 and 42 amino acids, respectively[9][119]. The APP intracellular domain (AICD) fragment produced simultaneously thus also changes size[120]. Interestingly, PSEN1 mutations generally increase steady-state $A\beta_{42}/A\beta_{40}$ isoform ratios, which is therefore now considered the relevant culprit by proponents of the amyloid hypothesis[58][121]. The longer amyloid isoforms have additional hydrophobic amino acids in their C-terminal part and are thus more hydrophobic and prone to aggregation[122][123], and accordingly also more toxic in cell viability assays as these features correlate[20][118][124].

The measured cell toxicity of Aβ oligomers is very structure-dependent[125][126][127], and it has not yet been possible to identify the specific disordered structural forms of the peptide that allegedly causes AD[128][129], although recently, specific structural features have been found to correlate with toxicity[124]. A number of mechanisms have been suggested[130], such as impairment of long-term potentiation[131] and oxidative stress caused by the peptides[132]. Aβ forms membrane channels that facilitate calcium transport[133][134][135][136]. The calcium dyshomeostasis thereby caused by Aβ is a potential mode of pathogenicity[29][30][135][137]. Aβ may also interrupt copper-mediated prion-protein interaction with NMDA receptors [138] and impair the respiratory chain in mitochondria[139][140][141][142].

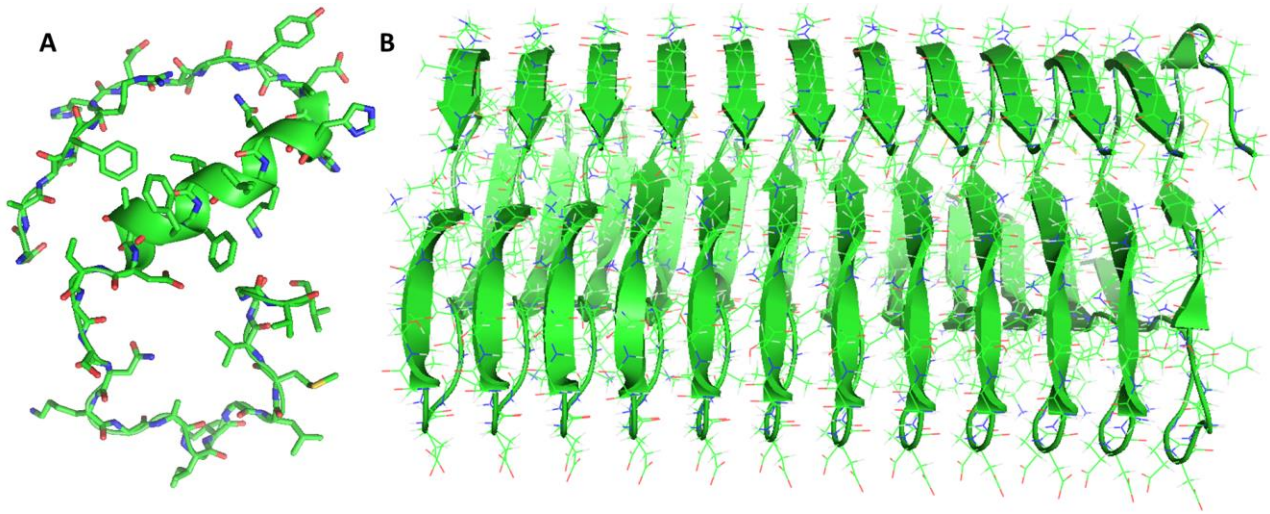

Figure 2. A) The disordered structure of monomeric $A\beta_{40}$ in water (PDB: 2LFM)[143]. **B)** The regular β-sheet structure of Aβ aggregates characteristic of senile plaques (PDB: 2MXU)[144] (Figures were produced using the Pymol software).

The quantitative amyloid hypothesis is thus being abandoned in favor of a "qualitative" gain-of-function hypothesis centering on the $A\beta_{42}/A\beta_{40}$ ratio[10][119]. As most FAD-causing mutations increase this ratio[145][146][147][148], it could indeed be pathogenic. The increase in this ratio is probably caused by the fact that γ-secretase cleavage of APP proceeds towards the N-terminal of Aβ in consecutive steps of 3−4 amino acids[149] and the FAD mutations are likely to affect the precision of this step-wise cleavage[150][151]. PSEN1 mutations tend to be dominant, i.e. heterozygote carriers are likely to develop AD, which has been taken to support a gain-of-toxic function as the compensatory presence of the wild type does not prevent disease[119].

The recent electron microscopy structure of γ-secretase shows details of PSEN1 having a transmembrane helix topology[152][153]. Although the loops are missing in the experimental structure, loops and other missing atoms have been included in full structural-dynamic models in physiologically

relevant membrane environments[154][155] (see **Figure 3A** and **Figure 3B** for full atomic structures of PSEN1 [155] modeled from the 2015 electron microscopy topology[153]). The interior space around the active site constituted by two catalytic aspartates is sensitive to long-range motions caused by dynamic membrane packing effects and loop motions that function as plugs in the transmembrane channel that open access to PSEN1's active site[155]. The gate-plug mechanism suggests that PSEN1 mutations impair membrane packing and thereby substrate selectivity by reducing the steric control of the catalytic site, leading to reduced cleavage precision and increased $A\beta_{42}/A\beta_{40}$ ratios[155]. This mechanism can be directly coupled to clinical outcome: Clinical age of symptom onset of patients carrying PSEN1 variants correlate significantly with increased polarity and loss of stability of the protein[156], consistent with PSEN1 membrane structural integrity as a determining feature of PSEN1-variant caused FAD.

In line with the amyloid hypothesis, drugs have been pursued that prevent the formation of amyloid oligomers, the assumed pathogenic species[157], either by direct molecular interaction with Aβ[158][159][160] or by inhibition of its production[161][162]. Given the potential mechanistic relationship between catalytic proficiency of γ-secretase and the $A\beta_{42}/A\beta_{40}$ ratio, inhibiting γ-secretase could create phenotypes that resemble the FAD-causing mutants; thus, it is hardly surprising that such inhibitors can produce adverse cognitive effects in human clinical trials[13][163]. Instead, *modulation* of the produced relative $A\beta_{42}/A\beta_{40}$ levels is now pursued as one of main clinical strategies in the form of so-called γ-secretase modulators[97][119][161].

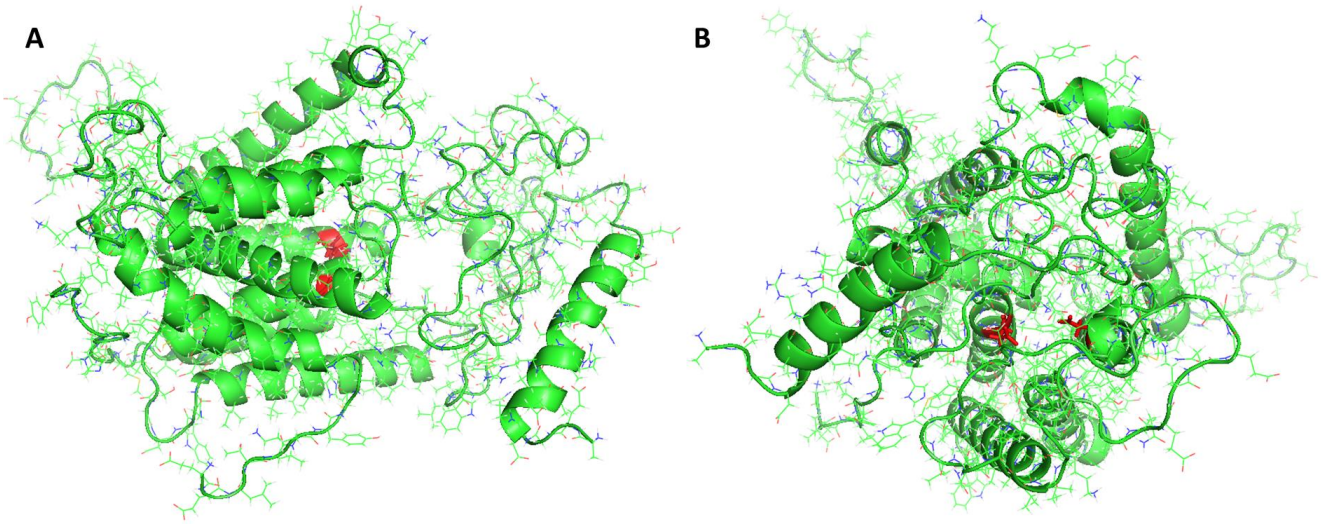

Figure 3. All-atom structure of mature presenilin 1 with loops from homology models and molecular dynamics simulations[155], based on the partial presenilin coordinates of the 2015 electron microscopy structure of γ-secretase[153]. **A)** Seen from the in-membrane perspective; **B)** seen from the above-membrane perspective. The catalytic aspartate residues D257 and D385 are shown in red. The loops play a major role in controlling access to the protein's interior.

Challenging the amyloid hypothesis

Recently, the $A\beta_{42}/A\beta_{40}$ ratio and specific chemical properties of PSEN1 mutants have been shown to correlate significantly with clinical symptom onset[156], providing a first relationship between human AD and protein-chemical features, which complements data from cell and mouse models that provide poor correlations to clinical data in meta-analysis[9] and in the transition to human trials[7][163]. Yet, correlation does not imply causation, and a complex disease such as AD features many apparent correlations. One example is the $A\beta_{42}/A\beta_{40}$ ratio: While this ratio *does* indeed correlate inversely with human symptom onset for the PSEN mutations[156], it does not for the phenotype-wise mixed APP mutations[9], and the increased ratio in PSEN mutant phenotypes could thus simply be a reflection of the loss of function of PSEN as the true cause of disease. Thus, all relevant correlations and equally

well all *lack* of correlations must be viewed in their totality. More generally, the following issues challenge the amyloid gain-of-function hypothesis:

First, it remains unclear how the curious $A\beta_{42}/A\beta_{40}$ ratio defines disease, while total levels of $A\beta_{42}$ do not (total levels tend to decrease or change insignificantly in PSEN1 mutant phenotypes[9][148]). Presumably, higher $A\beta_{42}/A\beta_{40}$ ratios either lead to more oligomers or impart them with more sinister properties, yet how can the ratio, but not $A\beta_{42}$ alone, produce such an effect? Lower overall production of Aβ caused by PSEN1 mutation does not necessarily imply lower steady-state levels of the presumed pathogenic oligomers, if oligomer clearance rates are lowered even more. Both $A\beta_{42}$ and $A\beta_{40}$ clearance has been found to be reduced by 30% in SAD, while production was unaffected[164]. This could imply that steady-state Aβ pools are increased in SAD, but this is inconsistent with the reduced levels seen in PSEN1 mutants[146]. Thus, it probably rather reflects the increase in degradation-resistant plaques, whereas intracellular soluble Aβ may have decreased: This would explain the lower clearance rates measured and reconcile the data discussed above.

The oligomers may be more degradation resistant than monomers, but probably less than the tightly bound β-sheets of plaques. The amyloid hypothesis does not explain how the PSEN1 mutant phenotypes with commonly observed lower steady-state levels of $A\beta_{42}$ could still lead to more pathogenic oligomers. In this author's view it would require that i) physiological oligomers are hetero-oligomers consisting of mixtures of $A\beta_{42}$ and $A\beta_{40}$; such hetero-oligomers have been studied by Pauwels et al.[165]; ii) local surplus of $A\beta_{42}$ seeds $A\beta_{42}$-enriched hetero-oligomers that may be more pathogenic and plausibly more resistant to degradation than oligomers of lower $A\beta_{42}$ content, so that the clearance is reduced to increase the steady-state pool of pathogenic oligomers. This competitive seeding mechanism could explain why a larger $A\beta_{42}/A\beta_{40}$ ratio, but not the total $A\beta_{42}$ levels, may cause disease.

Second, it is a shortcoming of the amyloid hypothesis that the *normal functions* of Aβ and APP and, for that matter the other APP products, sAPPα and AICD[120], are not accounted for: APP-related proteins are merely viewed as pathogenic, yet the elaborate splicing of APP is clearly there for a reason, and there is substantial support for a physiological role of Aβ[166][167][168][169]. In addition, AICD regulates transcription in a way that somewhat resembles that of the intracellular domain of Notch also produced by γ-secretase[170]. These functions of cleavage products further complicate the strict amyloid gain-of-function hypothesis[47]. Specifically for Aβ, concentrations below nano-molar (as encountered within cells) are neurotrophic whereas only high concentrations (typically micro-molar levels as seen in cell assays) are toxic[168][171]. *This suggests that there is a therapeutic window of Aβ levels that should be stabilized, rather than merely reduced.*

Third, current research models of the amyloid hypothesis rely on measurements of Aβ toxicities[172] and aggregation tendencies[173] occurring at micro-molar concentrations, corresponding to ~1 year of total brain production, 1000-fold higher than the biologically relevant concentrations[174][175]; yet AD is age-dependent and gradually develops over years. Some toxic modes associated with physiologically relevant concentrations have been reported[126][176], but the toxic mechanism of Aβ oligomers remains debated[130][177].

The amyloid hypothesis lends support from APP mutations that cause AD, and specific Aβ variations are widely heralded as confirming the amyloid hypothesis, a notable example being the recently identified protective A2T variant[178]. **Figure 4** shows the sites of Aβ mutation and cleavage in APP. Attempts to link clinical phenotypes (protective vs. pathogenic) to actual biochemical properties are being made, with particular focus on the effect on APP cleavage, and thus Aβ production, and aggregation tendencies[179]. However, data from APP mutant models are mixed[180][181][182]: Some cases show normal behavior despite high amyloid levels[183], while impairments resembling AD features can be induced by other features such as metabolic deficiencies[184].

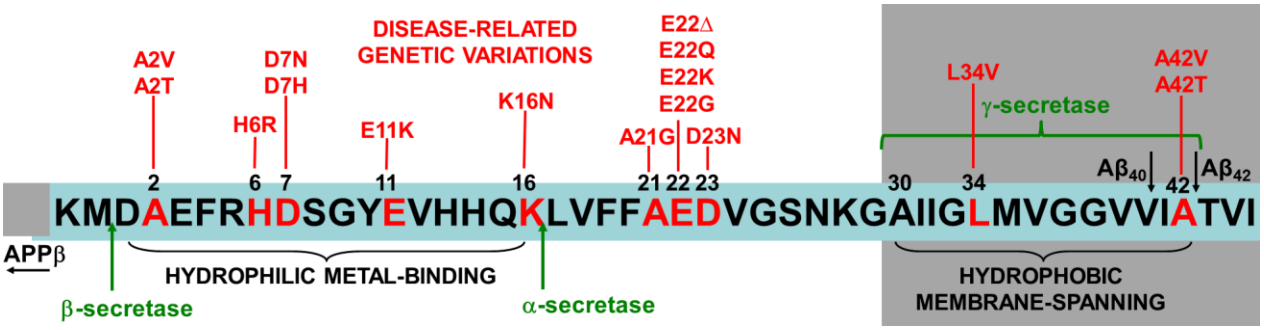

Figure 4. The Aβ region of APP. Cleavage by α-, β-, and γ-secretases is shown in green, and disease-related mutations are shown in red.

Furthermore, meta-analysis shows that reported biochemical data for Aβ variants are disturbingly heterogeneous: Published amyloid levels and $A\beta_{42}/A\beta_{40}$ ratios measured in cultured cells, toxicities from cell viability studies, and aggregation propensities from thioflavin T assays for these variants scatter to the extent that conclusions based on any one or even several of these variants are statistically meaningless and thus do not support the "quantitative" amyloid hypothesis[9]. Distinct toxicities of Aβ variants expressed in cell lines do correlate with their aggregation tendencies in *cell assays*[118][124]. Yet, while the relationship between cell toxicity and aggregation propensity is strong[118], these toxicity and aggregation data do not correlate to *human clinical data* of Aβ variant carriers at all[9]. Thus, at best, data from cell and mouse models are too heterogeneous to give insight into human disease, at worst, they are fundamentally unrelated to the disease[9].

Fourth, the APP mutations are even more problematic than the biochemical heterogeneity would imply: Different mutations in APP produce distinct histopathologies and different intensities of tangles and plaques as typified by cerebral amyloid angiopathy (CAA) vs. classical AD; some variants lead to increased, others to decreased total Aβ levels; variants such as A2V, H6R, D7N (using the Aβ numbering scheme) produce $A\beta_{42}/A\beta_{40}$ ratios similar to wild type APP, whereas APP variants E22G, E22K, and E22Q *lower* this ratio; and average clinical ages of symptom onset vary from the *thirties* to

the *sixties* of age[9]. *In conclusion, the biochemistry of APP mutations provides no evidence of gain of function and does not explain variations in clinical outcome.*

A fifth point of concern is that while it is thus increasingly clear from correlation studies that the Aβ variants tell us little about disease[9], the *PSEN1 variants* tell us a lot[156]. Many more mutations in PSEN1 than in APP cause AD, although APP contains the final Aβ product[59], and the PSEN1 mutations have chemical properties that have now been directly correlated to clinical FAD characteristics[156]. These PSEN1 mutations cause lower γ-secretase activity and reduced production of Aβ; this could suggest that PSEN1 is more central to AD than Aβ is[185]. The PSEN1 mutant phenotypes are much more clinically and biochemically homogenous than are the APP phenotypes, as they produce classical AD phenotypes and tend to increase $A\beta_{42}/A\beta_{40}$ ratios in most cases[186]. The $A\beta_{42}/A\beta_{40}$ ratios correlate with actual human clinical data[156]. Thus, a competitive seeding mechanism that would lead to gain of toxic oligomers *could* be a determinant of human AD as cell and mouse models do not capture such a mechanism. Yet, it could equally reflect loss of functional monomeric Aβ, to be explored below.

Sixth, the other important histopathological features of AD, e.g. metabolic deficiencies, massive post-translational modifications, metal ion imbalances, and oxidative stress, are not accounted for in any current version of the amyloid hypothesis [19]. It is unsatisfactory that the amyloid hypothesis does not explain the main risk factor of disease (age) as emphasized by e.g. the two-hit hypothesis[187]. The aging human proteome is *the* relevant framework for understanding AD. The aging proteome exhibits down-regulation of genes involved in synaptic function, including calcium homeostasis and signaling, and vesicular transport, and upregulation of anti-stress and anti-inflammation genes[38]. The amyloid hypothesis, founded very much on the FAD mutations in PSEN1 and APP, does not explain the vast majority of sporadic AD cases, where no such Aβ-related genes seem to be strongly involved.

Finally and most importantly, the amyloid gain-of-function hypothesis has so far failed utterly in its attempts to produce a cure or even halt of AD[7][11]. The clinical human data have not confirmed the supposedly promising data from cell and mouse models used to research the drug candidates of the main pipelines[13][163], consistent with the poor correlations between human clinical data and these types of data models that is already clear from meta-analysis[9]. In fact, clinical trials essentially disprove the quantitative gain-of-function hypothesis[6][12][188][189], but perhaps not a *qualitative* gain-of-function mechanism centering on specific local pools of $A\beta_{42}$-enriched hetero-oligomers. The following should be noted:

(i) *Active* Aβ immunization with AN1792 produced cases of brain inflammation and continued cognitive decline[190], and no such treatment strategy has produced favorable Phase 3 outcomes[180];

(ii) *Passive* Aβ immunization with Solanezumab showed no improvement in cognitive function in the two major phase 3 trials[191]; however subsequent analysis of the combined data revealed a positive effect on cognition[192][193]. Also, Bapineuzumab did not show any benefits[194] and can produce adverse effects vs. placebo[195] *despite* lowering amyloid levels[196]. Gantenerumab is currently being continued despite reports of inflammation[197]. Since the antibodies bind differently to Aβ, they may affect the functional monomer pool and the oligomer pools differently, and in principle, this may be a useful way to reconstitute functional loss of Aβ monomers.

(iii) Both the γ-secretase inhibitors Avagacestat and Semagacestat that reduce Aβ production have shown adverse cognitive side effects, and were discontinued[198][199]. While this has been interpreted as possibly due to impairment of Notch signaling and other adverse effects of reduced PSEN proficiency, the immunization data are more consistent with loss of Aβ function in the light that a therapeutic window of neurotrophic Aβ levels are well-established[168][171] and since these strategies do not impair other functions of PSEN1. Instead, *both* adverse clinical outcomes of passive immunization and γ-secretase inhibition can be explained by loss of function.

The gain vs. loss of function hypotheses are summarized in relation to some central data in Table 1. The possibility of a loss of function mechanism will be explored in detail in the remainder of this review.

Table 1. Observations related to AD can be explained both as gain or loss of Aβ function[a].

Observation	Explanation, gain of function	Explanation, loss of function
Aggregation	oligomers are toxic by some specific mechanism	oligomers represent loss of functional monomer
PSEN1 mutant phenotypes	high $A\beta_{42}/A\beta_{40}$ ratios increase Aβ oligomer pool or specific toxicity, even if total $A\beta_{42}$ levels decrease	less produced Aβ represents loss of function; however, increased oligomerization reduces functional monomer pool
APP mutant phenotypes	mixed phenotypes cannot be consistently explained. Some affect APP cleavage to increase, others to reduce Aβ levels	N-terminal mutants lose metal-binding; C-terminal and over-expressing mutants aggregate and lose monomer pool
Toxicity upon overexpression of FAD mutants	oligomers are toxic by some specific mechanism	depletion of metal status in cells
adverse effects of γ-secretase inhibitors	due to lack of selectivity and Notch impairment	due to reduced functional Aβ levels, as in PSEN1 phenotypes
adverse effects of passive Aβ immunization	not targeting the right form of Aβ	due to reduced functional Aβ levels
Positive effects of some Aβ antibodies	removing pathogenic oligomers	reconstituting functional monomers
Oxidative stress and metal dyshomeostasis	possible toxic modes of Aβ-metal interactions	directly related to APP/Aβ function (loss of Aβ function causes copper buildup in cells)

[a] See text for discussions and specific references.

Moving forward: What does the structural chemistry of Aβ peptides tells us?

While the heterogeneity in *clinical* data is due to disease risk modifiers, the substantial heterogeneity in *biochemical* data for Aβ variants, such as measured aggregation tendencies from thioflavin T assays, is due to different lab protocols and the special chemical features of Aβ[9]. Efforts are ongoing to produce consistent, stable, and reproducible monomeric and oligomeric Aβ samples that will remove some of the protocol-based heterogeneity[200][201][202]. However, even beyond the sample management issue, the structural variability of these highly disordered peptides[203] renders observed properties such as toxicity very conformation-dependent, and, since conformation is very dependent on chemical environment, *Aβ structures and their derived toxicities are highly sensitive to concentration, pH, ionic strength, co-solvents, and the time scale of the experiment performed*[118][204].

Identifying the physiologically relevant structures of the Aβ monomer is a necessary prerequisite to understanding both its oligomerization and potential gain of pathogenic action, but also its natural roles[9][20][123][205] such as membrane interaction[206][207]. In water, the free Aβ monomer has a disordered coil-dominated structural ensemble with some helix and little β-strand[203][208][209][210]; this is consistent with available nuclear magnetic resonance (NMR) and circular dichroism (CD) spectroscopic data on the monomer and reflected structural models with PDB codes 1BA4[211], 1IYT[208], 2LFM[143], 1Z0Q[209], and 1AML[212].

In co-solvents and close to membranes, the helix content of Aβ monomers increases[212], due to the helix dipoles being stabilized in low-dielectric environments[213]. The shape-shifting nature is illustrated by two representative structures deduced from NMR data in **Figure 5A**, showing the Aβ structure in water, and **Figure 5B** showing the structure of Aβ in a membrane-mimicking chemical environment; in the latter case, the C-terminal of Aβ has become an extended, potentially membrane-spanning helix, an observation that fits well with the widely documented membrane channel properties of Aβ[134][136][214].

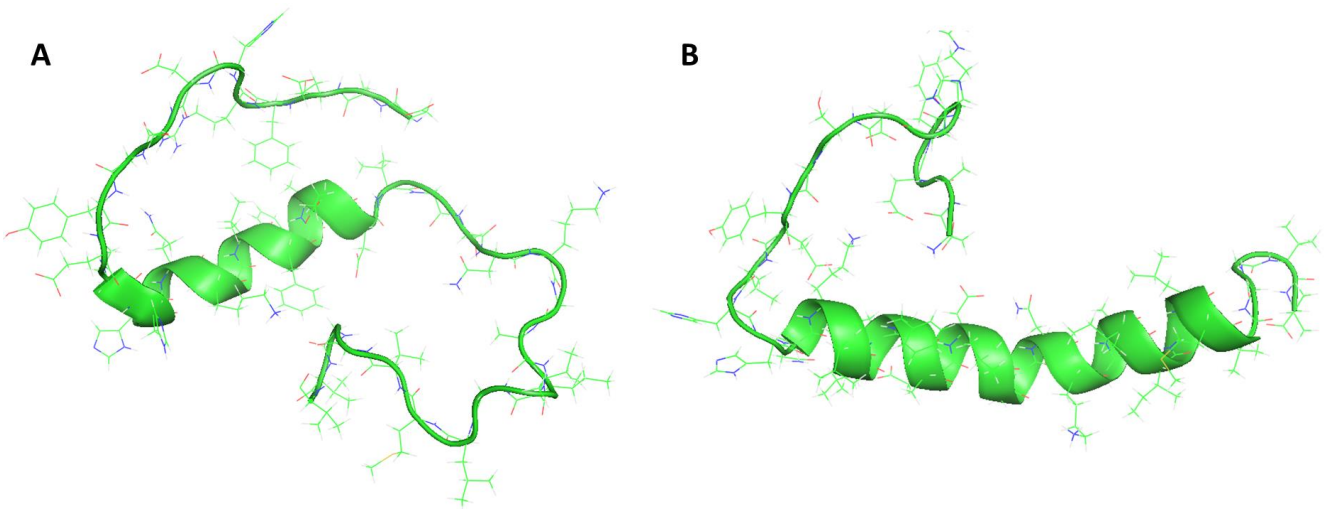

Figure 5. The shape-shifting nature of Aβ: **A)** NMR-derived structure of $A\beta_{40}$ in water (PDB: 2LFM)[143]. **B)** NMR-derived structure of $A\beta_{40}$ in a micelle membrane-mimicking environment (PDB: 1BA4)[211]. The hydrophilic N-terminal is shown on top, whereas the hydrophobic C-terminal, which forms helix in the membrane-environment, is shown to the lower right in both cases.

On the other hand, if the monomer Aβ is subject to other chemical interactions or modifications, such as high Aβ concentration, contact with metal ions[215][216][217][218][219] or genetic mutation[220][221], it forms oligomers and fibrillar aggregates. In these structures, the peptide converts structure from helix-coil to the extended sheets of β-strands found in senile plaques, as seen in **Figure 2B**[209]. As an example of the relevance of time scale, Abelein et al.[222] found mainly coil, some indications of helix, and no β-strand in the pure $A\beta_{40}$ monomer, but after days at room temperature they observed β-strand characteristic of aggregated peptide[222]; the fibrils making up the senile plaques consist mainly of such extended sheets, packed with metal ions[209][223][224].

Considering the major structural variability of Aβ occurring both *in vitro* and *in vivo*, attempts have been made to identify the "physiologically relevant" ensembles of the peptide, by correlating specific structures directly to human clinical outcome and cell toxicities. This provided the first statistically significant relationships between fundamental chemical properties and clinical and biochemical phenotypes[118][124][213]: Remarkably, hydrophobic exposure in the most disordered structures of Aβ correlate with features of the human disease, i.e. the diagnosis age of patients carrying a specific variant, whereas other structures do not. This could suggest that the coiled disordered structures with a small (<25%) helix segment are the physiologically relevant forms of Aβ[213].

The differences in experimental toxicities of genetic Aβ variants are well explained by each variant's conformational ensemble: Specifically, toxicity correlates with the extend of hydrophobic exposure of disordered structural parts of the variants, providing a detailed molecular picture with statistically significant correlations to experimental toxicity data[118][124]. Thus, Aβ aggregation and cell toxicity is caused by hydrophobic exposure in specific disordered amyloid states that can be targeted by molecular intervention, e.g. antibodies[158][160]. However, in the light of the poor correlation between toxicity assays and clinical disease features[9], the question still remains whether this oligomerization-driven cell toxicity has anything to do with AD.

Evidence for normal physiological functions of APP/Aβ

Mouse knock-out experiments indicate that APP-related proteins have physiological roles: APP-knockout alone reduces synaptic proliferation, whereas single-knockout of the related amyloid precursor-like protein 2 (APLP2) does not affect neuron function and morphology[225]. However, knockout of *both* APP and APLP2 simultaneously is lethal and indicates an important role of APP/APLP2[226][227] and that each protein can compensate the absence of the other[228]. Although these proteins are unimportant for cell differentiation[229], they affect synaptic plasticity under normal

conditions[230] and are essential for hippocampus function and spatial learning of mice[231]. The role of APP or its cleavage products is also seen from its importance in resisting kainite-induced seizures[232]. The APP family of proteins are mainly important in aged, not juvenile mice[227]. Consistent with these experiments, several roles of these proteins in maintaining synapses have been identified[233][234].

Aβ produced from APP also has a normal function in cells: The work by Yankner et al.[171] is often cited by proponents of the gain-of-function amyloid hypothesis as evidence of toxicity. However the work actually found beneficial effects of the peptide at lower concentrations, but toxic effects at higher concentrations[171]. Plant et al. have confirmed this by different lines of investigation, showing that inhibition or depletion of $A\beta_{40}$ by either secretase inhibitors or Aβ antibodies is lethal to cultured neurons, and $A\beta_{40}$ is protective in a concentration-dependent way, whereas $A\beta_{42}$ was not[235]. Aβ depletion also impairs neuronal activity in mice[166]. Important hints to the nature of this function comes from work showing that Aβ monomers protect against copper- and iron-induced toxicity[167] and play a natural role in regulation of vesicle release in hippocampal synapses, with more extracellular Aβ related to increased release probability[169].

These various findings indicate that loss-of-function is a real peril that must be accounted for, and the studies therefore anticipated the negative outcome of strict γ-secretase inhibitors such as Solanezumab ten years later[198]. At that time, the impaired proficiency and reduced Aβ levels of FAD-causing PSEN1 mutations were also well known, further mystifying the rationale behind the quest for strict Aβ containment. This is a remarkable example of the inability of scientific paradigms to rationally self-correct, but it also reflects negatively on the R&D management of major companies who were obsessed with the toxic side of the therapeutic Aβ window while ignoring the neurotrophic side; this has, unfortunately led to a waste of research (and shareholder) funds, and more importantly, to a delay in the testing of drug candidates that could be truly efficient[163][236]. *And still today, research*

and drug development efforts do not present a balancing strategy of Aβ, despite evidence summarized above that such a balance is necessary.

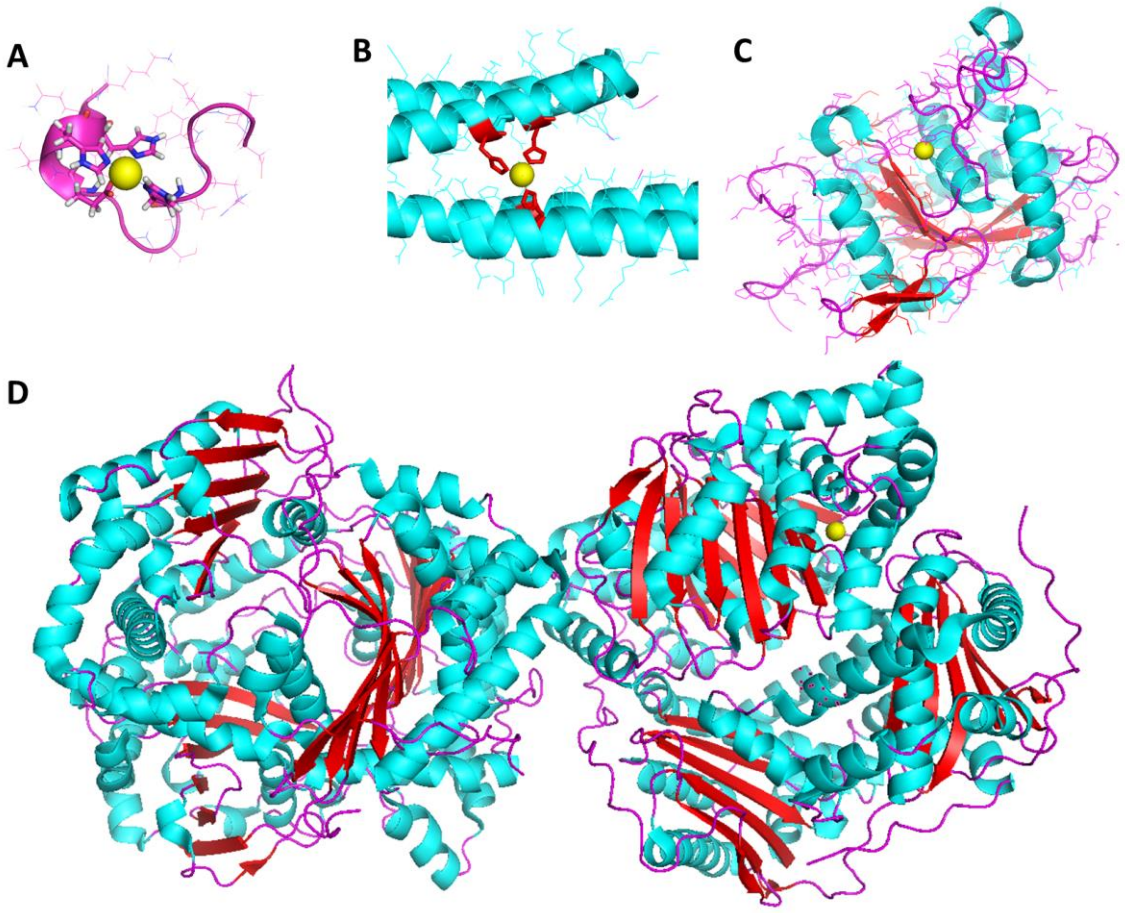

Figure 6. Zinc sites (zinc shown in yellow) in key proteins relating to Alzheimer's disease: **A)** Aβ based on the NMR structure 1Z09.pdb[237]; **B)** the crystal structure of the E2 domain of APP (3UMI.pdb)[238]; **C)** ADAM17 as a representative of the ADAM family of zinc peptidases with α-secretase activity, based on the structure 3LE9.pdb[239]; **D)** Loss of metalloprotein function contributes substantially to the pathology of Alzheimer's Disease. Insulin degrading enzyme is one of several Aβ-degrading zinc peptidases that will lose function upon metal dyshomeostasis (figure made from coordinates in the reported crystal structure 2WK3.pdb[240].

The natural function of APP: Metal binding and transport

In the following, it is argued that the normal physiological function of APP/Aβ is regulation of intra-neuronal relative copper levels via low-to-moderate affinity divalent metal transport. The theory that metal ion imbalances play a role in AD is well-established[19][34][46], and in particular calcium has been associated with AD for three decades[241] and reviewed in detail[28][29][135][137][242][243]. Most of the proteins central to AD have copper or zinc sites, with some examples shown in **Figure 6**[19][244]. Evidence for APP as a metal transport protein includes the following:

i) First, APP has multiple established metal binding sites[245][246][247][248]. The large extracellular part of APP is generally divided into two domains, E1 and E2 : E1 consists of the growth-factor like domain that contains a heparin binding loop [247] and the copper-binding domain (CuBD)[249][250] consisting of His151, His147, and Tyr168[249][251]. The affinity for copper is moderate, approximately 10 nM[246]. There is also a Zn(II) site within residues 181−200[252]. Several Cu(II) and Zn(II) sites are present in the E2 domain of APP-like proteins reported to regulate conformation and possibly function[238][253].

ii) APP reduces bound Cu(II) to Cu(I) in association with electron transfer and disulfide bond formation[254]. The combination of copper binding sites and this redox chemistry has been suggested to imply that APP transports Cu(I) across the membrane in analogy to other copper-transporting proteins having membrane-associated copper binding sites that reduce Cu(II) to the transportable Cu(I) oxidation state, e.g. the plasma membrane copper transporter Ctr1, which is cleaved from the membrane upon high copper status to prevent uptake of copper[255].

iii) Knockout of APP in cultured neurons leads to reduced Cu(I) induced toxicity in neurons[256], suggesting that APP is essential for neuronal copper uptake. In addition, overexpression of APP leads to intracellular copper deficiency and overexpression of the Aβ-containing C-terminal fragment of APP leads to both copper and iron deficiency in mouse brains[257]. Copper efflux function

due to APP can be reduced by copper chelating ligands[258]. The specific copper-binding domain of APP has been shown to reduce toxicity from copper exposure[259].

iv) Other researchers have found[260] that increased copper exposure in aged mice leads to Aβ accumulation and down-regulation of low-density lipoprotein receptor-related protein 1 (LRP1) – a transporter of Aβ [261] that also controls APP trafficking and processing[262][263].

v) Buxbaum et al. found 20 years ago that APP cleavage is regulated by increases in either protein kinase C or calcium levels independently[264]. Secreted forms of APP have been found to protect against excitotoxicity and reduce calcium levels in cells[265]. Addition of the 105-residue C-terminal fragment of APP to cultured cells has been found to increase intracellular calcium levels and make rat cortical neurons more prone to glutamate-based excitotoxicity; calcium levels could, interestingly, be reduced by cholesterol[266]. The C-terminal fragment is neurotoxic both with and without the Aβ sequence fragment included[267].

vi) APP stabilizes the Fe(II) exporter ferroportin and has been suggested to be analog of ceruloplasmin (a copper- and iron transport protein that interacts with ferroportin) although it does not have ferroxidase activity as initially suggested [268].

Aβ: A metal ion transport peptide

If APP is a membrane protein that controls certain aspects of intra-neuronal metal status, the question is, how does it do so? One answer to this question is by its intrinsic ability to bind and respond to metal ions within the membrane, as summarized above; the other is by the use of its splicing product, the Aβ peptide. The evidence for such a mechanism is now presented:

i) First of all, Aβ is itself a genuine metal-binding peptide with an archetypical N-terminal metal-binding site constituted by three histidines (His-6, His-13, and His-14 using Aβ numbering, see **Figure 4**)[269][270]. Metal ions such as Cu(II) and Zn(II) mostly form 1:1 complexes with Aβ,

sometimes with a secondary, low-affinity binding site[248][271][272][273]. The binding of metal ions to Aβ changes its structure and induces formation of fibrils[272][274]; these metal-rich fibrils resemble the senile plaques characteristic of AD.

ii) Furthermore, Aβ is also an archetypical *amphiphile*: It has a hydrophilic N-terminal region with established metal binding properties and a hydrophobic C-terminal region providing its membrane affinity[19]. This amphiphilic design seems ideal for micelle- or membrane-channel formation.

iii) As the apo-Aβ carries a charge of −3, binding of metal ions of positive charge +2 or +3 would increase Aβ hydrophobicity and most likely also its association with membranes[213].

iv) The Aβ monomer is disordered in aqueous solution but forms helix structures in low-dielectric environments such as co-solvents and micelles, thus providing a structural rationale for its membrane channel function[135][214][275][276], not simply as a toxic mode of Aβ action but as a potential natural function of the peptide.

In summary, the "shape-shifting" nature of Aβ, the increased helix forming tendency in membrane environments, its metal-binding properties, and the amphiphilic chemical features (hydrophilic N-terminal half, hydrophobic C-terminal half) indicate that the peptide has evolved to interact with metal ions and cell membranes as part of its natural function.

Indeed, such a function has been observed: Aβ forms channels that enable calcium transport through membranes[135][136][277][278][279], and these channels can be blocked by zinc[207][214]. Also, the loss of cellular copper causes copper depletion in the highly abundant cytosolic anti-oxidant protein SOD1 that plays a central role in amyotrophic lateral sclerosis (ALS) which shares many similarities with AD[19][280][281]; dietary copper compensates this loss and reduces Aβ production[282].

Regulation of the APP/Aβ metal transport system: Enter secretases

As evident from the research summarized above, APP and Aβ are genuine metal proteins and metal peptides, respectively, having strong association with membranes and direct effects on intra-neuronal metal status. However, the mechanism by which such an APP/Aβ system senses and regulates metal status remains to be explained. I argue here that this regulation mechanism works via the much studied (in the different context of amyloid production within the amyloid hypothesis) splicing of APP by the α-, β-, and γ-secretases.

i) First, β-site APP cleaving enzyme 1 (BACE1), the aspartyl protease that initiates production of Aβ by cleaving its N-terminal from APP[283], contains a copper site, and overexpression of BACE leads to reduced SOD1 activity consistent with loss of intracellular copper (copper availability is required for proper holo-protein SOD1 folding and activity)[284][285]. This suggests that BACE activity somehow reduces copper status in neurons. Hou et al. showed that not only does copper exposure lead to increased APP expression and Aβ formation, it does so by action of a copper sensing protein (CUTA) that directly interacts with the β-cleavage site of APP[286], and recently, Multhaup's group showed that the transmembrane region of BACE1 contains a genuine, highly conserved cysteine- and methionine-rich copper binding site, which loses copper binding affinity upon mutation of cysteine to alanine[287]. Supplementing this evidence, Gough et al. showed that N-terminal archetypical metal-binding site of Aβ, which is also the β-cleavage site, constitutes the actual site that regulates β-secretase cleavage of APP[288]. These various data strongly suggest that BACE1 is activated by copper levels by specific copper binding to facilitate cleavage of APP.

ii) A variety of metal chelators reduce amyloid plaque formation[289], down-regulate APP expression and lower Aβ secretion as a response to the depletion of the intracellular free copper pool[290]. Strong metal chelators such as EDTA reduce γ-secretase activity and thereby, production of

Aβ, consistent with a reduction of intra-neuronal metal status directly down-regulating Aβ-producing γ-secretase activity[291]. Zou et al. found that the beneficial effect of monomeric Aβ in protecting against metal-induced oxidative stress toxicity resembles that of classical non-natural metal chelators such as EDTA[167].

iii) Inhibition of copper transporters increase Aβ in brain lysates and decrease expression of Aβ-degrading proteases [292].

iv) Copper-induced toxicity in rabbits leads to learning impairment and to enhanced Aβ plaque formation, suggesting that production of Aβ from APP increases with metal status[293].

A consistent explanation of these observations is that high copper status is counteracted by activation of the amyloid-producing secretases, leading to cleavage of APP and production of the copper-transport peptide Aβ, which is then exported out of neurons to produce the metal-enriched plaques that characteristically define AD in human brains. *Thus, APP/Aβ is probably a neuronal metal transport system, with the spliced Aβ peptide working as an exporter in combination with the low-density lipoprotein receptor family of proteins. The widely studied cleavage of APP by α-, β-, and γ-secretases to produce Aβ is therefore suggested to be a regulation mechanism of neuronal metal status.* It is notable that by analogy, the plasma membrane copper transporter Ctr1 is also cleaved from cell membranes: Its cleavage is a result of high copper load, to prevent copper uptake[255].

Observing the chemical composition of senile plaques, with their massive co-location of copper, zinc, and iron ions[209][223][224], they might be referred to as amyloid-metal deposits rather than simply amyloid deposits. These plaques are non-pathogenic[107][108], and the aggregation propensity of amyloid variants does not correlate with disease severity[9]. Why then do we see this buildup of plaques if not as part of the pathogenesis itself? The alternative answer is: Either as an irrelevant downstream consequence of the true pathogenesis, or due to a protective mechanism related to disease. Within the view presented here, these metal-enriched plaques are a natural result of excessive metal

export from the neurons via Aβ, a metal-binding amphiphilic membrane-associated peptide plausibly evolved specifically for this purpose.

Accordingly, the ability of metal ions to bind and induce Aβ aggregation[19] [46][217][218][219][294], which has so far been interpreted as a *toxic mechanism* of metal-Aβ interaction, can instead be explained as a physiological metal-Aβ interaction and metal-export-deposit function. The observations of beneficial effects of metal chelators on Aβ status in cultured cells[295][296] and in *Drosophila* that expresses human Aβ[297], and on the cognition of mice[298] as a result of the reduced intracellular copper levels and concomitant reduced Aβ production, are all in line with the regulation mechanism suggested above.

Aggregation causes loss of functional Aβ monomer

It may seem ironic to a community accustomed to the gain-of-toxic-function amyloid hypothesis to think of the opposite scenario, loss of normal Aβ function. However, the main observations in support of gain of function are equally consistent with loss of function, whereas various other observations are *mainly* consistent with loss of function.

First of all, accounting for the normal beneficial functions of $A\beta_{40}$[166][167] should be expected even of a gain-of-function mechanism, yet the gain-of-function hypothesis does not do so, despite the established normal functions. Gain of function is usually attributed to the PSEN1 and APP genetic mutations because they are mostly autosomal dominant[299][300]. The gain of function is supposed to arise from an increase in quantity or malicious quality of Aβ oligomers by means of aggregation processes, consistent with mostly autosomal dominant mutations[117][133]. As the total Aβ pool consists of innocent, functional monomers and pathogenic non-functional oligomers, oligomerization implies a depletion of the pool of functional Aβ monomers concomitant with an increasing oligomer pool. The dominant FAD mutations can therefore equally well cause disease by

loss of Aβ function, since the heterozygote's wild type Aβ cannot protect itself against aggregation and thus, depletion of the overall pool of Aβ monomers.

Loss of Aβ monomers is consistent with the fact that *total* Aβ levels decrease in most FAD-causing mutants[146][185], whereas gain of function requires a specific mechanism driven by increased local $A\beta_{42}/A\beta_{40}$ ratios, as discussed above (*competitive seeding*). Most of the genetic risk factors of FAD are now known to reduce soluble monomer $A\beta_{40}$ levels either directly[185] or by increased aggregation: $A\beta_{40}$ production is in some cases halved by PSEN1 mutations [301], and if the $A\beta_{42}/A\beta_{40}$ ratio causes aggregation, this would equally constitute a substantial loss of the normal functions of Aβ[302]. Typical Aβ deposits in senile plaques correspond to perhaps 5−10 years of total Aβ production, or about seven years calculated recently[105]. Rather than implying, as commonly done, that these plaques represent a *toxic overload* of Aβ, clearly, the documented functions of Aβ will be affected by such a massive deposition.

Metal homeostasis and AD

Dyshomeostasis of calcium, zinc, iron, and copper levels is an established pathological feature of AD[19][31][34][46][216][243][303], as probably first suggested in the case of zinc by Burnet[304]. The massive metal-enrichment in senile plaques[46][270][305] was a hint of this, but it is more specifically seen from upregulation of metal transport proteins in APP/PSEN1 mutant expressing mice[306][307] and upregulation of zinc transporters[306][308] and metallothioneins[309] in AD patients. The heterogeneous data on metal status in AD brain parts can be reconciled by a mechanism whereby metal ion status is perturbed from the *bound* pool in proteins to the *free* pool in the cytoplasm; this shift has many biochemical consequences[19].

While metal imbalances could be a consequence of general neurodegeneration and thus not causative, there is support for a causal role[19][46]: One example is the "zinc cascade", zinc's control

over the Aβ balance via zinc peptidases involved both in Aβ formation and degradation[19]. Cleavage of APP at the α-cleavage site, which lies within the Aβ region and thus prevents amyloid production, is catalyzed by membrane zinc-proteases of the ADAM family (A Disintegrin And Metalloprotease family)[310][162] (**Figure 4C**). Aβ is rapidly degraded by a number of proteases[311], notably neprilysin[312][313], angiotensin-converting enzyme[314], matrix metalloproteases[315][316][317], and insulin degrading enzyme[318][319] (**Figure 4D**). As previously pointed out[19], *all of these are, curiously, zinc proteases.* Impairment of some of these proteases can be related to AD[317]. If zinc dyshomeostasis manifests as loss of functional protein-bound zinc pools, these zinc enzymes would be impaired, providing a direct causal relationship between zinc homeostasis and amyloid balance[19].

The homeostasis of the three divalent metal ions calcium, copper, and zinc is tightly controlled due to the narrow window between nutritional value in important proteins and toxic nature in free form[320]. Dependent on their mutual concentrations, they serve in neuron signaling, apoptosis, inflammation, oxidative stress control, and cell proliferation: In particular, life-death processes of cells are controlled by calcium[321][322][323] and zinc levels[324][325][326], and zinc plays a central role in the apoptosis, oxidative stress, immune defense, neurogenesis, and synaptic plasticity[327][328]. Calcium-regulated life-death choices work through transcription factors such as MEF2[322], which are also risk factors of AD recently identified from GWAS[73].

Their homeostasis is intimately linked and synergistic[329]: The zinc-regulated life-death choices are regulated by oxidative stress and are coupled to copper levels and metallothioneins[330]. Increased cytoplasmic zinc levels elevate calcium levels and activate calcium-dependent signaling pathways that lead to death[331]. Notably, zinc also inhibits Notch signaling[332], which modulates cellular life-death decisions *via* zinc finger transcription factors, and this mechanism provides one of several pathways that unify SAD and FAD, with PSEN1 phenotypes having genetically impaired Notch

signaling due to PSEN1 mutation, where zinc overload provides a sporadic counterpart of impaired Notch signaling.

The metal ions need to be treated differently and are heterogeneously distributed in the brain[333]: Copper is redox-active and binds more strongly to the nitrogen donor ligands of APP and Aβ, which can be explained from the Irving-Williams series of chemical binding constants[19]. Copper is particularly enriched in the hippocampus where AD typically starts[334]. In contrast, calcium and zinc are redox-inactive but calcium is oxophilic and preferably binds to oxygen-donor ligands, whereas zinc also binds nitrogen- and sulfur-donor ligands; they also differ in terms of cellular distribution and abundance: Calcium is most abundant, whereas zinc is abundant in vesicles of zinc-enriched neurons, transported by ZnT3 transporter proteins[335][336], and zinc is deposited in AD patients together with plaques, around impaired blood vessels, and in cells with tau pathology[337], with significantly increased levels in hippocampus of AD patients[338].

Copper is used in active sites of enzymes such as cytochrome c oxidase of the mitochondrial respiratory chain and SOD1[19]. Loss of functional copper would lead to impairment of the respiratory electron chain and mitochondrial energy inefficiency, increased oxidative stress and potentially apoptosis of the neuron; thus metal dyshomeostasis can cause mitochondrial dysfunction characteristic of neurodegenerative diseases[19]. SOD1 is located on chromosome 21 like APP and plays a key role in zinc/copper homeostasis and oxidative stress control in the vulnerable neurons with their high oxidative metabolism[339]. Mutations in SOD1 is a major genetic risk factor of familiar ALS[340][341]. Another gene on chromosome 21, Down syndrome critical region gene 1, produces cognitive deficits and inhibits Calcineurin's calcium-dependent phosphatase function[342], which contributes to the neurological impairment characteristic of trisomy 21. Copper dyshomeostasis is well known to be neuropathological as evident from e.g. Menkes and Wilson's diseases[343][344].

Based on the available evidence summarized so far, it can be suggested that *APP/Aβ controls secondary metal level balances, notably the Cu/Zn ratios, and, in combination with presenilins, the Cu/Ca ratio.* This is consistent with the moderate affinity of the metal sites in APP[246] and Aβ[19]; metal transporters generally separate into high- and low-affinity types based on their role, and low-to-moderate affinity seems suitable for ratio maintenance, whereas high-affinity transporters control primary import and export[345]. The APP/Aβ is thus most likely a secondary metal transport system with metal ion selectivity: For example, copper is known to bind 2−3 orders of magnitude more strongly to Aβ than zinc[19]. *This implies that the main function of APP/Aβ is to balance relative intra-neuronal metal levels.*

The proposed function of APP/Aβ and its loss-of-function role in AD is supported by the findings that presenilins induce copper and zinc uptake[346], while they leak calcium[347][348]. This indicates that presenilins are involved in maintaining Cu/Ca and Zn/Ca ratios. APP's established dimerization equilibrium and its proteolytic processing[349] may thus be a mechanism to control Cu/Zn and Cu/Ca levels in neurons; this dimerization of APP and associated Aβ production is facilitated by copper levels[350]. The processing of APP by presenilin-containing γ-secretase has recently been suggesting to be directly coupled to channel formation in presenilin, so that APP cleavage and channel activity may be linked[155].

As discussed above, there is evidence that metal binding at the archetypical N-terminal Aβ site within intact membrane-bound APP triggers APP cleavage and production of Aβ. Possibly, although speculative, the formed metal-Aβ peptides then use the instantaneously formed pores in presenilin to immediately exit the membrane after they have been produced by APP cleavage. This mechanism would explain why Aβ cation-selective channels are inhibited by zinc[206][207][214], an unusual property if not enabled to ensure metal ion selectivity of the APP/Aβ system. This "made-to-go"

mechanism would provide a pathway for extracellular deposition of metal-enriched senile plaques and explains why zinc exposure increases Aβ production from APP and leads to Aβ deposits[351].

CAA vs. AD: An explanation based on normal Aβ function

An important piece in the puzzle is the observation that genetic Aβ variations (see **Figure 4**) located within the N-terminal 1−16 region tend to give rise to classical AD phenotypes, whereas mutations within the C-terminal part of Aβ beyond position 20 cause CAA, e.g. the Dutch, Italian, Flemish, Arctic, and Iowa variants[9]. Also, all the FAD-related APP mutations that increase total amyloid levels are located in the N-terminal half of the peptide[9]. No one has provided an explanation for these differences. The common argument from the amyloid gain-of-function paradigm is that these mutants affect α-, β-, and γ-cleavage of APP in different ways: Clearly, the mutants are located in the neighborhood of these APP cleavage sites, and the Swedish double-mutation at the β-site (but outside the Aβ sequence) supports this view as it leads to large quantities of Aβ[352]. Others emphasize the changes in aggregation behavior of the produced Aβ variants themselves, as e.g. the highly aggregation-prone Arctic E22G variant[353][354].

As explained above, neither of these two features can explain the pathogenicity of APP variations: The $A\beta_{42}/A\beta_{40}$ ratio increases significantly vs. wild type in only two variants, D7H and E11K[9]. Total Aβ levels increase in A2V, D7H, E11K, K16N, and A21G, all in the N-terminal half. The assumed protective A2T variant reduces Aβ levels possibly by modifying the β-cleavage site[355], as the Swedish mutant. However, the pathogenic Osaka E22Δ variant also reduces Aβ levels, and the English H6R, Tottori D7N, Arctic E22G, Italian E22K, and Dutch E22Q do not change Aβ levels significantly[9]. If overproduction of Aβ (despite unaltered ratios) by A2V and the Swedish mutation should cause AD, it would be by a mechanism distinct from most APP and PSEN1 mutations. Yet,

researchers curiously continue to discuss models of AD, such as the Swedish model discussed below, as if overexpression of Aβ due to altered APP cleavage is a central feature of the disease.

If altered APP cleavage cannot explain the FAD/CAA mutants of APP, then, if we rely on the amyloid hypothesis, the changed product has to be pathogenic in those other cases where Aβ itself is changed without affecting β-cleavage, i.e. instead of levels (quantitative gain of function) it must be properties (qualitative gain of function). As discussed previously, there is no indication that the aggregation propensities, as presumably related to disease within the gain-of-function paradigm, has anything to do with disease: Many variants do have higher aggregation propensity than the wild type Aβ, which is rationalized by their tendency to increase hydrophobic exposure or reduced hydrophilicity[118]. However, there is no positive relationship between disease severity and aggregation tendency, in fact perhaps even the opposite: The four clinically most severe variants, A21G and A2V, E22Q, and E22Δ (age of symptom onset from 36−52 years) have *smaller* tendencies to aggregate than some other variants such as the English and Tottori variants with 56−61 years of onset[9], i.e. aggregation is not pathogenic and perhaps even protective.

Instead, the increased amyloid production seen in cultured mutant cell models resulting from mutation in the N-terminal of Aβ can be explained as a changes in metal-binding properties, with metal-binding ligand residues such as histidine, aspartate, and glutamate being changed in these mutations[269][356]. The question then emerges whether metal binding is impaired only in the free produced Aβ, or also in APP prior to APP cleavage, if the same site is accessible in the membrane-bound APP before cleavage. Copper increases APP expression and Aβ formation *via* CUTA interaction with the β-cleavage site of APP[286]. The N-terminal metal site of Aβ, which is also the β-cleavage site, constitutes the actual site that regulates β-secretase cleavage of APP[288]. It is notable that Cu(II) and Zn(II) bind differently to Aβ, and Cu(II) binds more strongly[19][357], so binding of the two metal ions may also have different effects on APP cleavage.

If the metal binding capacity of Aβ is reduced by direct mutation, as is the case in N-terminal FAD mutants such as D7H[358] but not C-terminal CAA mutants such as A21G[359], it would reduce the peptide's normal function of metal export, to cause the observed cell toxicity. *This mechanism would reconcile FAD-causing N-terminal Aβ variants with the more common PSEN1 phenotypes that cause depletion of functional Aβ by reduced catalytic cleavage of APP.* Currently, the amyloid hypothesis does not reconcile these phenotypes, because the APP variants do not have the same general tendency of increasing the $A\beta_{42}/A\beta_{40}$ ratio as the PSEN1 mutants do[9]. It explains why the very different phenotypes of APP variants can all cause disease, which has not previously been explained: Notably, N-terminal Aβ variants that increase amyloid levels have impaired metal binding function, whereas the remaining variants increase aggregation propensity or affect APP cleavage by γ-secretase.

The coupling to presenilin

Any theory of AD pathology must explain not only the Aβ imbalances but also the important role of PSEN. As discussed above, almost 200 PSEN1 mutations and a smaller amount of PSEN2 mutations cause early-onset FAD; within the gain-of-function amyloid hypothesis, these are thought to be pathogenic by their effect on APP cleavage, which generally leads to an increase of the observed $A\beta_{42}/A\beta_{40}$ ratio[97][119]. However, PSEN1's own functions also receive attention[59][60][303], and γ-secretase processes more than 20 other substrates, including Notch[360]. The number of PSEN1 mutations vs. APP mutations and the distinct functions of PSEN1 outside the γ-secretase, among other facts, have inspired a hypothesis that AD is due to PSEN1 loss of function[59]. The most established function of PSEN is its involvement in calcium homeostasis, as summarized by the following observations:

i) PSEN1 is homologous to calcium transporters and has a ion-channel-like topology[243][361]. Recently, the structure of PSEN1-containing γ-secretase has been elucidated in substantial detail: The

3-dimensional structure of human γ-secretase was determined using single-particle cryo-electron microscopy[153][362], with PSEN having nine transmembrane helices resembling a barrel-like domain protecting the active site where two aspartates cleave APP in the membrane[153][360]. PSEN1 can work within the γ-secretase complex or without it[363][364].

ii) Consistent with these structural features, PSEN1 works as a calcium channel independently of its role in γ-secretase activity[361][347][365]. Although a calcium-leak effect in the endoplasmic reticulum (ER) has been disputed by at least one group[366], the role of PSEN1 in calcium homeostasis of the ER is established[303][367][368]. PSEN1 mutations impair calcium homeostasis and show elevated calcium levels upon exposure to Aβ[348], and soluble APPα (sAPPα) fragment produced from APP restores calcium homeostasis and rescues PSEN1 mutant-exposed cells from apoptosis[369].

iii) PSEN1-knockout also causes loss of copper and zinc uptake and subsequent deficiency of these metal ions in brains of mice, leading to loss of superoxide dismutase 1 (SOD1) activity [346], the main antioxidant cytosolic copper-zinc protein that is also the main genetic risk factor of familial ALS [370][371].

One of the main processes of cell turnover is the lysosomal degradation of cell parts including aggregated proteins in the cytoplasm by autophagy[372]; this clearance process is necessary for neurons to survive and is impaired in AD[367][373][374][375]. PSEN1 is important for lysosomal protein degradation and autophagy, which is impaired by FAD-causing PSEN1 mutations[376][377]. At the molecular level, PSEN1 helps to fold and glycosylate vacuolar ATPase that is essential for lysosome acidification and thus, activation of autophagy[376]; there is substantial evidence for a link between lysosomal failure in AD and calcium dyshomeostasis caused by PSEN1 dysfunction, as recently reviewed[367].

The role of apolipoproteins in copper/zinc homeostasis

An accurate etiology of AD should also explain the important risk factor associated with the isoform 4 of apolipoprotein E (ApoE4). Relevant to the presented hypothesis, González et al. found that serum levels of zinc, copper, and insulin are significantly higher in ApoE4 allele carriers[378]. Zinc deficiency also reduces expression of apolipoproteins[379], whereas copper deficiency has been found in several studies to increase apolipoprotein levels[380][381]. In transgenic mice carrying the ApoE4 isoform, tau aggregation was modulated by zinc, i.e. zinc and ApoE4 work together to produce tau pathology[382]. These results show, from various points of view, important crosstalk between ApoE proteins and zinc and copper levels.

Also of interest in this context, clusterin (apolipoprotein J) has been shown to be upregulated upon zinc exposure[383]; this observation potentially relates clusterin, a risk factor of AD[69], to zinc-dependent aging regulation. Clusterin has been found to work as a chaperone that regulates the turnover of copper ATPases that are central to brain copper homeostasis[384], as shown in several neurological diseases but perhaps best in Wilson's disease where mutations in these copper proteins directly cause disease[385]. Such mutations have indeed recently been related to ApoE4, as carriers of this isoform show earlier onset of Wilson's disease[386]. A consistent explanation of the various findings summarized above is that the lipoproteins are involved in the metal-transport function of Aβ.

The Swedish mutation and why mice do not develop AD

Whereas loss of function accounts for the therapeutic window of Aβ, the PSEN1 phenotypes and adverse outcomes of clinical Aβ-containment strategies, the gain-of-function amyloid hypothesis seems more consistent with the fact that some AD-causing APP mutations, notably the Swedish K670M/N671L double mutant[387], increase steady-state Aβ levels multiple times compared to wild type APP[388][389], potentially by enhancing the β-cleavage vs. α-cleavage of APP[352]. The

Swedish mutation was widely heralded as support of the early "quantitative" cascade hypothesis and is still used as a model of AD in mice[390][391].

However, several other AD-related APP mutations produce similar or reduced Aβ levels vs. wild type and heterogeneous phenotypes, and the $A\beta_{42}/A\beta_{40}$ ratio does not generally increase[9][388]. This makes the Swedish mutation phenotype-wise, and in terms of chemical effect on APP cleavage, very distinct from other APP mutations. Furthermore, the curious double-mutation has only ever been identified in a few families, making the genotype extremely rare. The combined rarity of phenotype and genotype renders the widespread use of this mutant as a transgenic mouse model of AD curious considering the amount of both APP and PSEN1mutant phenotypes that actually lower Aβ levels. *The Swedish mutation increases total Aβ levels, but does not change the ratio, whereas most other mutants related to AD do the exact opposite.*

Yet, the Swedish mutant does produce cognitive deficits when overexpressed in mice, how can this be reconciled with the hypomorphic PSEN1 phenotypes? One reasonable answer is that the Swedish mutant causes Aβ overproduction that causes the adverse effects by depleting intracellular metal status. Yankner et al.[171] already showed that there is a therapeutic window of Aβ concentrations. In this light, it is curious that noone has considered the possibility that the seemingly contradictory phenotypes of PSEN1 and Swedish mutants could be reconciled by this therapeutic window: In the former case, Aβ is depleted either by lowered production or increased oligomerization, in the latter case it is over-abundant and potentially aggregating, putting Aβ levels easily outside the healthy range in both cases. The suggested metal transport function rationalizes the observation of this this window.

Plaque load is substantially reduced if the zinc transporter ZnT3 is knocked out, showing that plaque formation depends on the function of zinc transport[392]. In contrast, ZnT3 knockout by itself gives age-dependent cognitive deficits in mice[308]. Overexpression of this mutant reduces SOD1

activity, a sign of copper deficiency, which could be remedied by copper exposure, showing that the Swedish mutant has a specific adverse effect on copper homeostasis in cells[282]; this effect can explain why the Swedish mutant gives AD in a way consistent with other mutants but unrelated to the high production of Aβ; i.e. while the APP cleavage is affected, other properties of the protein that relate to its copper homeostasis are affected too.

Why do cell/mouse FAD models give different results from human patient clinical trials? If the herein stated mechanisms are true, cell toxicity commonly measured upon expression of FAD mutants in cultured neurons and mice is due to extensive Aβ-metal export during overexpression of APP mutants and PSEN1 mutants. Incidentally, the normal wild-type mouse Aβ contains three different amino acids within the metal binding N-terminal: R5G, Y10F, and H13R. The host wild type Aβ is less toxic and less aggregation-prone than human Aβ and will modulate the amyloid pathology of such models unless it is knocked out[393]. Mouse Aβ still binds copper but differently from human Aβ[394]. It has been found that while copper binding and reduction is impaired in mouse Aβ due to these substitutions, the oxidative stress inducing effects have been found to be similar[395].

According to the arguments presented here, the transgenic mouse models relying on mutant overexpression cause Aβ production that lead to intra-neuronal metal depletion and impairment of the ability of synapses to maintain Cu/Zn/Ca gradients. In contrast, *in actual humans suffering from FAD, the APP copy number is stable over long periods of time* and lead to *impaired* metal export competence, because both PSEN1 mutants and APP mutants display reduced metal export capacity, and this causes *intra-neuronal metal overload*. This explanation is supported by the observation that zinc overload increases the extend of APP cleavage and extracellular metal-enriched Aβ deposits[351]; an impaired APP/Aβ system would be less capable of exporting metals and this would undermine Cu/Zn and Cu/Ca ratios, in particularly at high age when the brain is more exposed to oxidative stress and metal dyshomeostasis, according to quantitative proteomics studies of the aging human brain[38].

These explanations are consistent with the mechanism suggested above, reconcile cell/mouse vs. human data, and link PSEN1 calcium transport function to APP/Aβ.

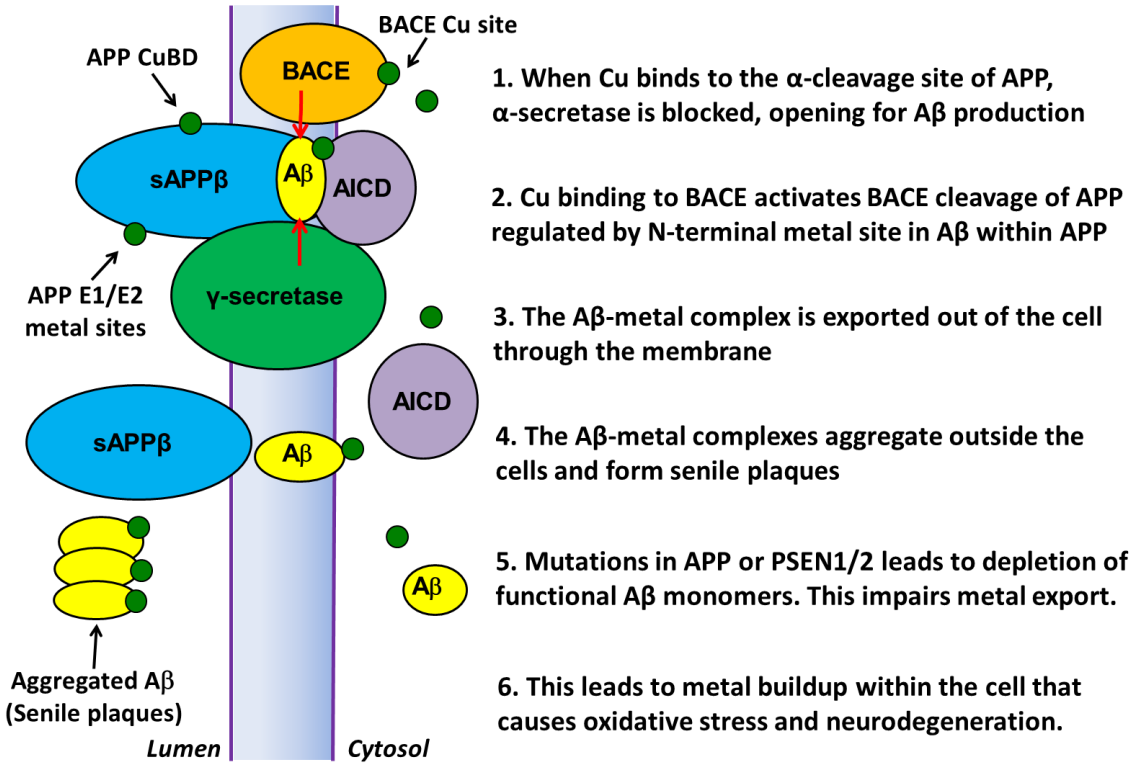

Figure 7. APP cleavage and neuronal metal transport: A suggested mechanism of function.

The tau connection

As mentioned, filaments of hyper-phosphorylated tau, i.e. neurofibrillar tangles, constitute a prevailing histopathological feature of AD[23][41]. Normally, the protein associates with the microtubules and maintain cytoskeleton integrity. However, the phosphorylation is related to loss of tau from microtubules and formation of structures that are seen as tangles[396][397]. It is not clear how tau pathology relates to amyloid pathology, although various links have been suggested. However, aggregation of tau is, as most other proteins, triggered by post-translational modifications: As has been

recently shown, zinc-tau interactions fundamentally determine tau phosphorylation and tau toxicity[398], and free zinc induces tau phosphorylation[399] and aggregation[400]. Therefore, tau pathology can be an early feature of metal dyshomeostasis[46][382], and tau hyperphosphorylation and aggregation may increase as cytoskeletons are dismantled during controlled cell death in the stage of neurodegeneration in a deteriorating vicious cycle of increased oxidative stress, metal imbalances, tau and amyloid pathology that reinforce each other[19].

Loss of function is consistent with Aβ-related biomarkers of disease

Two of the five most commonly used biomarkers of AD relate directly to Aβ, i.e. the measurement of $A\beta_{42}$ levels in the cerebrospinal fluid, and Positron Emission Tomography (PET) Aβ imaging[111]. However, the idea that these biomarkers relate to gain of toxic Aβ is an assumption, and below it is argued that these biomarkers more suitably indicate loss of Aβ function.

Amyloid deposits build up gradually over many years in AD[401], and positive PET imaging of Aβ deposits usually accompanies AD diagnosis[18][402]. However, as discussed above, plaque deposits are no longer considered the pathogenic feature of AD, both because many cognitively normal people have such deposits[108][109][110], because deposition does not correlate with cognitive decline[106][107], because aggregation tendencies of Aβ variants do not correlate with clinical severity[9], and because plaques, which are extracellular insoluble deposits of Aβ, are not very toxic, whereas intracellular soluble oligomers of Aβ are[112][113][114][115][116]. In contrast, a high plaque load may in some people indicate protective ability to export pathogenic forms of Aβ from cells[117][118]. The deposits may still represent pre-clinical disease states[111], and the PET imaging will continue to provide valuable information on amyloid balance, but its relationship to amyloid gain of toxic function etiology is not evident. Instead, since these deposits are not molecularly active inside the neurons, they primarily indicate that neuronal Aβ balance is disturbed, or more specifically, I argue

that they indicate a *depletion* of functional monomers inside neurons as a result of depositing of Aβ in plaques.

Similarly, reduced $A\beta_{42}$ in the cerebrospinal fluid is a useful biomarker of AD that correlates strongly to positive PET imaging discussed above[111]. In early-onset FAD, $A\beta_{42}$ levels were reduced already 25 years before cognitive symptoms[403]. The inverse correlation between $A\beta_{42}$ in the cerebrospinal fluid and extracellular insoluble plaque deposits from PET imaging[404] strongly indicates that the soluble pools of $A\beta_{42}$ have been depleted in favor of the deposits; since the deposits are not pathogenic, depletion of soluble $A\beta_{42}$ seems consistent with loss of soluble Aβ from the neurons, i.e. if the overall soluble pool of functional Aβ has been depleted in favor of non.functional oligomers and aggregates. In contrast, surplus of soluble intracellular $A\beta_{42}$ oligomers as argued by the more recent versions of the gain-of-function hypothesis is not directly commensurable with this depletion, because as oligomers belong to soluble pools, they would be expected to be in equilibrium with cerebrospinal fluid levels. The temporal ordering of biomarker output[111][405] is thus consistent with a gradual loss of the functional monomer pool, caused by increased demand for the peptide due to neuronal age-dependent stress, including metal dyshomeostasis, and due to the removing of monomers by continuous Aβ aggregation over time.

Relation to the main clinical outcome, memory loss

How does the suggested APP/Aβ divalent metal transport system relate to neuronal signaling and memory loss? As has been known for 25 years, AD is particularly characteristic by the loss of *synapses* rather than merely neurons[406][407]. The cholinergic hypothesis has for many years reflected the direct impairment of forebrain pathways of the acetylcholine-rich neurons involved in attention and working memory[49][50] but was challenged by its weaker association with histopathology and genetic

risk factors[408]. The evidence for a physiological function of the APP/Aβ system in signaling is as follows:

First, APP localizes in the pre- and postsynaptic compartments and is expressed in glutamatergic neurons, and NMDA receptor activation reduces Aβ production by activation of α-secretases, the zinc peptidases that cleave APP within the Aβ region to prevent Aβ production[114][409] (different previous results could be explained by transcriptional regulation upon prolonged NMDA receptor stimulation[409]). NMDA receptor activation is characterized by the channeling of metal ions through the neuron membrane, directly relating Aβ to this function that is essential for synaptic plasticity and memory[410][411].

Second, synapses are targeted by Aβ[412] and synaptic structure is affected by Aβ[413]; this could be a mode of toxicity, as has been suggested[414], but given the peptide's natural functions, *co-location with synapses could equally well be a result of Aβ's normal function*. This is supported by findings that Aβ regulates vesicle release in hippocampal synapses, with extracellular Aβ proportional to release probability[169]. Again, the concentration range in vesicles becomes critical, as the therapeutic window between neurotropic and toxic features is narrow[171].

These findings are consistent with the function suggested above, i.e. that Aβ is involved in divalent metal cross-membrane transport during neuronal signaling, as also implied by the findings of Ramsden et al.[276][415]. This function would immediately explain the need for a delicate balance of Aβ levels, as argued by Pearson and Peers[168], as such a balance parallels the delicate homeostasis of metal ions: Indeed, Abramov et al. found that short-term synaptic facilitation required balanced Aβ levels, as impairment resulted from both too high and too low levels of Aβ[169]. Synaptic activity requires the repeated flow of glutamate, calcium, and zinc in order to enable neuron signaling and memory formation [19][28][279][328], providing the rationale for such a function of Aβ in synapses.

Oxidative stress, proteostasis, metabolism, and mitochondria

Oxidative stress is a general feature of AD; most affected tissue bears signs of massive oxidative stress in the form of oxidized proteins and lipids[48][416]. Oxidations of proteins also affect protein turnover by modifying the surfaces of proteins and subjecting them to proteolysis[417]. The importance of protein turnover in AD and related neurodegenerative diseases is evident not just from the protein misfolding characteristic to these diseases, but also from risk factors relating to protein quality control[129]. It is notable that calcium play a key role in proteostasis regulation, and several regulators of proteostasis work by inhibiting calcium channels to decrease cytoplasmic calcium levels, which upregulate protein quality control machinery[129].

Protein misfolding also plays a main role in ALS, although the pathogenic mechanism of the misfolded proteins remains unclear[418]. However, recently, it was shown that survival times of patients carrying a mutation in SOD1 correlate inversely with the energy cost of turnover of the particular SOD1 mutant[281]. This energy increase is typically caused by loss of stability and increased aggregation due to mutation or post-translational modification, but also depends on protein copy number and specific turnover costs that may play out within the RNA pool[419]. This suggests that neurodegenerative diseases may be caused by neuronal energy deficiency at least partly owing to increased protein turnover; if so, misfolded proteins do not cause disease by some molecular toxic mode, but rather indirectly via their burden on systemically available neuron maintenance energy[281].

AD has been viewed by some as a mitochondrial disease[139][140][141][142][420], and Aβ can accumulate in mitochondria and interact with inner mitochondrial membranes where neuron energy is produced[139]. As discussed above, the evidence of Aβ as a small metal-binding[19][31][248] membrane-interacting[135][207] peptide seems strongly supported by its chemical amphiphilic nature and its conformational features[9][213]. Aβ causes permeabilization of cell membranes by channel formation[133][135][277][278][279]. The resulting calcium dyshomeostasis is massively

documented[29][30][137][242][421]. If APP/Aβ controls secondary metal ion balances, then its impairment would compromise metal homeostasis. Metal ion dyshomeostasis is a two-edged short in terms of energy: Loss of functional bound metal pools undermines the proteostatic machinery and prevents refolding of metalloproteins, which increases proteome maintenance costs due to increased protein turnover[281]. In addition, the imbalances in metal pools will most likely increase ion-pump energy costs as well. Protein turnover and ion gradient maintenance are the two main energy requiring processes of cells[419]. The increased demand for neuronal energy will put strain on mitochondria and cause pathological changes, increased ROS production, and plausibly, once the neuron maintenance energy requirements can no longer be met, apoptosis of the neurons in question. These neurons are, incidentally, often the most energy demanding and metal-enriched cells of the hippocampus where AD often initiates.

Thus, it is not surprising that neurons, among the most energy-requiring cells of the body, are particularly sensitive to protein misfolding, oxidative stress and metal dyshomeostasis: In the "energy hypothesis" of neurodegerative diseases[281], the known metabolic features of these diseases can be coupled to the protein misfolding and explain why these diseases occur specifically in neurons, where allocation of energy to ion pumps is particularly required. Neuronal exhaustion will be endangered by chemical aging, as seen in the upregulation of proteins relating to exactly anti-stress function, calcium-regulation, and fatty acid metabolism in aging human brains[38]. Such pathology would be fueled by metal ion dyshomeostasis caused by ineffective APP/Aβ/PSEN1 divalent metal transport resulting from FAD-related mutations, both in terms of the increased cost of ion pumping and because metal dyshomeostasis increases misfolding of metalloproteins central to AD[19]; this is possibly the reason why APP and PSEN mutations cause AD in earlier age as the energy threshold that triggers neuron death will be reached earlier.

Thus, SAD and FAD can be unified within the loss of function mechanism. In contrast, within the amyloid hypothesis, it is unclear how SAD arises by accumulation of pathogenic Aβ oligomers and how aging triggers AD.

Perspectives on diagnosis and treatment of AD

AD needs to be diagnosed as early as possible to enable timely treatment that can prolong life-span[403]. At the time that patients are diagnosed, disease progression already prevents major improvements in prospects[37][403][422]. As argued previously[19], since this disease represents an imbalance gradually aggravated by aging processes, the only viable biomarkers are abundance *ratios* that quantify relative levels of the chemical species involved in disease progression. Markers of accelerated chemical aging such as e.g. early oxidative stress or lipid metabolism should be combined with markers such as $A\beta_{42}/A\beta_{40}$, $Cu/A\beta_{40}$, and $Ca/A\beta_{40}$. Also, tissue-specific Cu/Zn and Cu/Ca vs. healthy controls may be relevant markers of the metal dyshomeostasis that is suggested to cause AD.

In addition to the development of effective early biomarkers based on chemical aging and metal homeostasis perturbations, a treatment should address the imbalances described in this review. Given the presented mechanism of disease, it is not particularly surprising that Mediterranean diets and active life styles reduce risk of AD[84][91][92]. Obviously, such risk modifiers should be actively applied in treatment strategies; anything else would be neglect of the available tools to secure optimal patient life quality. While we do not know the active ingredients in all cases, a healthy and varied diet with vegetables, olives, and fish, green tea, coffee and curry is known to reduce AD risk[92][423]. Such a diet should be complemented by effective medicine if possible, which, according to the mechanisms suggested in this review, should have as a strategy to *rebalance* Aβ levels rather than *deplete* them entirely; the latter strategy has so far failed, as discussed above. Based on the evidence provided here, Aβ balancing would be a key aspect of a treatment strategy. It could further be combined with anti-

inflammatory hormone treatment and possibly additional zinc and anti-oxidant vitamin supplements. When combined with a carefully monitored treatment strategy pursuing an active cognitive life-style, this would, in this author's opinion, provide the best possible multi-factor treatment strategy of this terrible disease, although much research will clearly be needed to identify the most relevant compounds in such a strategy.

Concluding remarks

In this review, the anomalies and inconsistencies of the current version of the amyloid hypothesis have been addressed in an attempt to provide a fully consistent etiology of AD. This etiology centers on the identification of the normal physiological function (a natural role has already been implied by many lines of research) of the APP/Aβ system and PSEN all related to maintaining relative metal ion levels within the neuron. These metal balances, notably the Cu/Zn and Cu/Ca ratios, are clearly affected by these proteins as seen from multiple studies. Impairment of the function of these proteins is then argeud to be the cause of early-onset familial AD, and this enables a unification, not only of the amyloid, oxidative stress, and metal ion hypothesis of AD, but also of the sporadic and familial forms of disease, as discussed in the latter part of the review.

The loss-of-function etiology of AD provides solutions to inconsistencies that are not currently accounted for by the gain-of-function amyloid hypothesis that dominates the field. It also has substantially more explanatory power while preserving the role of Aβ aggregation, not as a pathogenic mode of action, but as a loss of the functional Aβ monomers. The gain-of-function amyloid hypothesis, without a function clarified, provides a link to the genetic risk factors APP and PSEN1 and PSEN2, but not to the main genetic risk factor of SAD, ApoE4, and the main risk factor of AD, age. With a function clarified, all these features can be understood in context. Notably, the amyloid deposits often taken to indicate overload are interpreted instead here as a sign of intracellular functional monomer

deficiency. To prove this theory, the extracellular and intracellular Aβ pools must be separated into their various types, to test whether Aβ levels are indeed deficient inside neurons of people with AD. The loss of function hypothesis explains clinical failures of Aβ reducing drugs and why inhibition or depletion of $A\beta_{40}$ by either secretase inhibitors or Aβ antibodies is lethal to cultured neurons[235], and the findings that Aβ depletion impairs neuronal activity in mice[166]. Thus, new drug strategies should center on balancing and reconstituting functional monomers rather than strict reduction.

The synthesis provided in this review is not intended to be final; nor is it anywhere close to providing a full detail of the biochemical complexity of this major disease. However, it is the hope of the author that the proponents of different theories on the etiology of AD will see this attempt at a synthesis as a welcome bridging between camps and fields; given the complexity of this disease, such bridging is urgently needed, as is the working together of scientists across disciplines, if we are ever to conquer and defeat this terrible form of dementia that affects so many millions of people today.

Conflicts of interest: The author has no conflicts of interest to declare.

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